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The effect of acute angiotensin II blockade on renal function in rats with reduced renal mass

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The effect of acute angiotensin II blockade on renal function in rats with reduced renal mass. The effect of acute Ang II blockade on renal function in rats with reduced nephron number was assessed in micropuncture studies. The Ang II receptor blocker, losartan, was administered at a dose of 10 mg i.v. at two intervals following five-sixths renal ablation. At eight weeks following ablation, Ang II blockade (Ang IIX) increased sodium excretion [$U_{Na}V$, Ang IIX 2.2 ± 0.4 μEq/min; time control (TC) 1.0 ± 0.3 μEq/min; $P < 0.05$] but did not reduce mean arterial pressure ($\overline{AP}$, Ang IIX 142 ± 6 mm Hg; TC 151 ± 6 mm Hg), glomerular transcapillary pressure (ΔP, Ang IIX 50 ± 1 mm Hg; TC 50 ± 1 mm Hg), or urine albumin excretion ($U_{Alb}V$: Ang IIX 149 ± 18 μg/min; TC 168 ± 20 μg/min). Similarly, at two weeks following ablation, Ang II blockade increased $U_{Na}V$ (Ang IIX 2.8 ± 0.4 μEq/min; TC 0.5 ± 0.2 μEq/min; $P < 0.05$) without reducing $\overline{AP}$ (Ang IIX 132 ± 6 mm Hg; TC 140 ± 7 mm Hg), ΔP (Ang IIX 50 ± 3 mm Hg; TC 48 ± 2 mm Hg), or $U_{Alb}V$ (Ang IIX 32 ± 3 μg/min; TC 36 ± 10 μg/min). These findings indicate that within the remnant kidney, Ang II promotes sodium retention but does not have an acutely reversible effect on glomerular pressure or permselectivity.

Reduction of renal mass leads to hyperfiltration and hypertrophy of remnant nephrons. When accomplished by partial renal infarction in the rat, reduction of renal mass also leads to increases in systemic and glomerular capillary pressure [1–3]. Previous studies have suggested that angiotensin II (Ang II) contributes to these hemodynamic changes and also to the later development of glomerular injury in the remnant kidney. Anderson et al [1] found that sustained inhibition of Ang II converting enzyme normalized systemic and glomerular capillary pressure in rats subjected to renal ablation. Subsequent studies have shown that Ang II receptor blockade has the same effect as converting enzyme inhibition in this model [4]. Treatment with an Ang II receptor blocker for one week has also been shown to reduce systemic pressure, glomerular pressure, and albumin excretion rate in renal ablated rats which were maintained without treatment until remnant glomerular injury was established [5]. Together, these findings suggest that endogenous Ang II increases systemic and glomerular pressure following renal ablation and promotes albumin filtration in injured remnant glomeruli. The purpose of the current study was to identify the mechanisms by which Ang II contributes to these changes. Angiotensin II has multiple effects on the kidney. In normal rats, infusion of Ang II increases sodium reabsorption, increases systemic blood pressure and glomerular capillary pressure, and reduces the glomerular ultrafiltration coefficient [6–9]. Each of these effects of exogenous Ang II can rapidly be reversed by Ang II receptor blockade. Two previous studies have examined the effect of Ang II blockade or remnant kidney function [10, 11]. In one study, a peptide Ang II receptor blocker reduced glomerular capillary pressure and single nephron GFR while the glomerular ultrafiltration coefficient remained constant [10]. In another study, both a peptide Ang II receptor blocker and a converting enzyme inhibitor reduced glomerular capillary pressure and increased the glomerular ultrafiltration coefficient while single nephron GFR remained stable [11]. The current study was performed to re-examine the effects of Ang II blockade on remnant kidney function using a recently developed non-peptide Ang II receptor blocking agent [12].

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Methods

Male Munich Wistar rats with initial weights of 280 to 320 g were subjected to five-sixths renal ablation by right nephrectomy and ligation of arterial branches supplying two thirds of the left kidney. They were maintained on standard chow containing ~24% protein by weight. Micropuncture studies were carried out in separate groups of rats at eight to twelve weeks and at two weeks after ablation. At each interval, one group of renal ablated rats received the non-peptide Ang II receptor antagonist losartan while a second group of renal ablated rats served as time controls ($N = 6$ to 8 rats in each group). Twenty-four-hour urine protein excretion was measured prior to micropuncture in rats studied at eight to twelve weeks after ablation so that rats could be divided into groups with similar glomerular injury. Additional studies were performed in intact male Munich Wistar rats to confirm that the dose of losartan used was sufficient to block the effect of Ang II.

Micropuncture protocol

Rats were anesthetized with Inactin (100 mg/kg body wt i.p.) and placed on a temperature-regulated table. A PE-50 tubing catheter was inserted in the right femoral artery and used for subsequent blood sampling and estimation of mean arterial pressure ($\overline{AP}$). $\overline{AP}$ was continuously monitored with an electronic transducer connected to a direct writing recorder. After tracheotomy, PE-50 catheters were inserted in the right and left jugular veins for infusion of rat plasma, saline, and radiolabled inulin. A PE-10 catheter was installed into the left ureter for collection of

urine. Plasma was infused in an amount equal to 1% body weight over 40 minutes, followed by a reduction of the infusion rate to 0.4 ml/hr for the duration of the study. Saline was infused at 1.2 ml/hr throughout the study. After 100 minutes, tritiated *methoxy*-inulin was added to the saline to achieve an infusion rate of ~40 μCi/hr following a loading dose of ~40 μCi. Clearance measurement were begun after ~120 minutes and carried out over two or three 30 to 40 minute clearance periods. In each period, a 200 μl arterial blood sample was obtained for determination of hematocrit and plasma inulin and protein concentrations. A renal vein blood sample was obtained with each arterial blood sample for determination of filtration fraction by renal vein inulin extraction.

In rats studied at eight to twelve weeks after ablation, baseline measurements of whole kidney function were made during the first 30-minute clearance period. Time control rats then received 1 ml of normal saline infused i.v. over 10 minutes followed by a 10-minute equilibration period and Ang II blockade rats received 10 mg of losartan in 1 ml of normal saline infused i.v. over 10 minutes followed by a 10-minute equilibration period. Measurements of whole kidney function were then repeated during two further clearance periods during which micropuncture measurements were performed. Timed (4 min) samples of tubule fluid were collected from surface proximal convolutions of three nephrons for determination of single nephron glomerular filtration rate (SNGFR). Time averaged hydraulic pressures were measured in surface glomerular capillaries, proximal tubules, and efferent arterioles with a continuous recording, servo-null micropipette transducer system (Model V, Instrumentation for Physiology and Medicine, San Diego, California, USA). The same protocol was used in rats studied at two weeks after ablation except that additional measurements of glomerular capillary and proximal tubule pressure were made during the baseline clearance period.

The efficacy of the dose of losartan used was assessed by measuring the pressor effect of a bolus dose of Ang II, 50 ng i.v., at the end of micropuncture studies. Further confirmation of the efficacy of losartan was obtained by assessing renal function before and after losartan in normal rats receiving a continuous infusion of exogenous Ang II. These studies were performed in a separate group of four intact male Munich Wistar rats weighing 290 to 320 g. Rats were prepared for micropuncture as described above except that a continuous infusion of Ang II, 150 ng/min was begun at ~60 minutes after anesthesia and continued throughout the duration of the study. Beginning at ~120 minutes after anesthesia, baseline measurements of whole kidney function and glomerular and proximal tubule pressure were made during a 30-minute clearance period. Losartan, 10 mg i.v., was then infused according to the same protocol used in renal ablated rats, and measurements of whole kidney function and glomerular and proximal tubule pressure were repeated during an a further clearance period.

Analytical

The albumin excretion rate was determined by rate nephelometry (Beckman Immunochemistry Analyzer) using anti-rat albumin antibody (Cappel Laboratories, Malvern, Pennsylvania, USA). The protein concentration of arterial plasma (C_A) was determined by refractometry. The radioinulin content of plasma, urine, and tubule fluid was assessed by liquid-phase scintillation counting.

Calculation and statistics

Efferent arteriolar protein concentration was calculated from the relation:

$$C_E = \frac{C_A}{(1 - FF)} \quad (1)$$

Oncotic pressure (π) of efferent and afferent arteriolar plasma was estimated from plasma protein concentration as

$$\pi = 1.629C + 0.294\,C^2 \quad (2)$$

A standard mathematical model was used to derive the glomerular capillary ultrafiltration coefficient (K_f) [13]. The statistical significance of differences among values of individual parameters before and after losartan or normal saline infusion was assessed by paired *t*-test and differences between values in losartan treated and time control rats was assessed by unpaired *t*-test. Significance in both cases was defined as $P < 0.05$. Values are expressed as means ±1 SE throughout.

Results

Studies at eight to twelve weeks

Time control and Ang II blockade rats were matched for body weight (TC 335 ± 6 g; Ang IIX 331 ± 6 g), weight gain following ablation (TC 36 ± 5 g; Ang IIX 31 ± 6 g), and 24-hour urine protein excretion (TC 189 ± 18 mg/day; Ang IIX 214 ± 37 mg/day) prior to study. Mean values for hematocrit, plasma protein concentration, mean arterial pressure under anesthesia and the determinants of single nephron GFR are summarized in Table 1. Values for hematocrit (Hct) and plasma protein concentration (C_A) were similar in the two groups. Baseline values for arterial pressure, GFR, and filtration fraction (FF) were also similar in the two groups. As expected both groups of remnant kidney rats exhibited baseline elevation of arterial pressure (TC 148 ± 8 mm Hg; Ang IIX 135 ± 7 mm Hg) and reduction of GFR (TC 0.72 ± 0.07 ml/min; Ang IIX 0.73 ± 0.10 ml/min) below levels observed in intact rats [3]. Infusion of 1.0 ml of normal saline did not alter values for these parameters in time control rats. Micropuncture measurements following saline infusion in time control rats revealed remnant nephron hyperfiltration (SNGFR 124 ± 6 nl/min) with values for single nephron plasma flow (Q_A 428 ± 27 nl/min), glomerular transcapillary hydraulic pressure (ΔP 50 ± 1 mm Hg) and the glomerular ultrafiltration coefficient (K_f 0.091 ± 0.010 nl/(s · mm Hg)) similar to those previously observed in remnant kidney rats at twelve weeks after ablation [4]. Infusion of losartan had no effect on arterial pressure in Ang II blockade rats ($\overline{AP}$ 142 ± 6 mm Hg post-losartan vs. 137 ± 7 mm Hg baseline). Infusion of losartan did cause a small increase in GFR (0.79 ± 0.10 ml/min post-losartan vs. 0.73 ± 0.10 baseline) but did not reduce ΔP below the value observed in time control rats (50 ± 1 mm Hg post-losartan vs. 50 ± 1 mm Hg time control post-saline). The absence of a difference in ΔP in losartan treated and control rats reflected the absence of differences in glomerular capillary pressure P_{GC} (64 ± 1 mm Hg post-losartan vs. 65 ± 1 mm Hg post-saline) and proximal tubule pressure P_T (14 ± 1 mm Hg post-losartan vs. 15 ± 1 mm Hg post-saline) in the two groups. Values for urine flow rate, sodium excretion, and albumin excretion are summarized in Table 2. Baseline values for each of these

Table 1. Glomerular hemodynamic function at eight to twelve weeks

		Hct %	C_A g/dl	$\overline{AP}$ mm Hg	GFR ml/min	FF %	SNGFR nl/min	Q_A nl/min	P_{GC} mm Hg	P_T mm Hg	$\overline{\Delta P}$ mm Hg	K_f nl/(s^{-1} · mm Hg)
Time control	baseline	45 ± 1	5.9 ± 0.1	148 ± 8	0.72 ± 0.07	0.30 ± 0.01						
(N = 7)	post	44 ± 1	5.7 ± 0.1	151 ± 6	0.74 ± 0.07	0.30 ± 0.01	124 ± 6	428 ± 27	64 ± 1	14 ± 1	50 ± 1	0.091 ± 0.010
Ang II blockade	baseline	45 ± 1	5.8 ± 0.1	135 ± 7	0.73 ± 0.10	0.30 ± 0.02						
(N = 7)	post	45 ± 1	5.7 ± 0.1	142 ± 6	0.79 ± 0.10[a]	0.28 ± 0.02	128 ± 5	431 ± 37	65 ± 1	15 ± 1	50 ± 1	0.078 ± 0.005

Abbreviations are: Hct, hematocrit; C_A, afferent arteriolar plasma protein concentration; $\overline{AP}$, mean arterial pressure; GFR, glomerular filtration rate; FF, filtration fraction; SNGFR, single nephron glomerular filtration rate; Q_A, glomerular plasma flow rate; P_{GC}, glomerular capillary pressure; P_T, proximal tubule pressure; $\overline{\Delta P}$, mean glomerular transcapillary hydraulic pressure difference; K_f, glomerular capillary ultrafiltration coefficient. Data are mean values ± SE.

[a] $P < 0.05$ post vs. baseline

Table 2. Urine sodium and albumin excretion at eight to twelve weeks

		Urine flow rate μl/min	Urinary sodium excretion μEq/min	Urinary albumin excretion μg/min
Time control	baseline	15 ± 3	1.0 ± 0.3	180 ± 33
(N = 7)	post	16 ± 3	1.0 ± 0.3	168 ± 37
Ang II blockade	baseline	16 ± 2	1.0 ± 0.3	148 ± 18
(N = 7)	post	22 ± 3[a]	2.2 ± 0.4[a,b]	149 ± 18

[a] $P < 0.05$ post vs. baseline

[b] $P < 0.05$ Ang II blockade vs. Time control

parameters were similar in the two groups, with heavy albuminuria in each group characteristic of remnant glomerular injury. No change was observed in urine flow rate, sodium excretion, or albumin excretion in time control rats. Ang II blockade caused a significant increase in sodium excretion (2.2 ± 0.4 μEq/min post-losartan vs. 1.0 ± 0.3 μEq/min baseline) without altering albumin excretion (149 ± 18 μg/min post-losartan vs. 148 ± 18 μg/min baseline).

Studies at two weeks

Additional studies were performed at two weeks after ablation to determine if Ang II blockade has effects at this interval which are no longer present at eight to twelve weeks after ablation. Time control and Ang II blockade rats were again matched for body weight (TC 288 ± 7 g; Ang IIX 285 ± 4 g). Rats lost weight over the two weeks after ablation, but the weight loss was not different between the two groups (TC 12 ± 5 g; Ang IIX 18 ± 5 g). Mean values for hematocrit, plasma protein concentration, mean arterial pressure and the determinants of single nephron GFR are summarized in Table 3. Overall, the results were similar to those obtained at eight to twelve weeks. Baseline values for arterial pressure, GFR, and filtration fraction were similar in the two groups. Both groups of remnant kidney rats exhibited baseline elevation of arterial pressure (TC 137 ± 8 mm Hg; Ang IIX 136 ± 7 mm Hg) and reduction of GFR (TC 0.70 ± 0.06 ml/min; Ang IIX 0.82 ± 0.03 ml/min) below levels observed in intact rats. Infusion of 1.0 ml of normal saline did not alter values for these parameters in time control rats. Micropuncture measurements following saline infusion in these animals revealed remnant nephron hyperfiltration (SNGFR 77 ± 6 nl/min) and hyperperfusion (Q_A 273 ± 34) which were slightly less prominent than at eight to twelve weeks following ablation. Glomerular transcapillary hydraulic pressure averaged 48 ± 2 mm Hg and K_f averaged 0.055 ± 0.005 nl/(s · mm Hg). Infusion of losartan had no effect on arterial pressure in Ang II blockade rats (132 ± 6 mm Hg post-losartan vs. 136 ± 7 mm Hg baseline). As at eight to twelve weeks, infusion of losartan caused a small increase in GFR (0.91 ± 0.04 ml/min post-losartan vs. 0.82 ± 0.03 baseline) but did not reduce ΔP. The post-losartan ΔP value of 50 ± 3 mm Hg in Ang II blockade rats was not different from the baseline ΔP value of 50 ± 1 in the same group or from the post-saline ΔP value of 48 ± 2 mm Hg in time control rats. Constancy of ΔP following Ang II blockade with losartan reflected a small, similar increase in P_{GC} (68 ± 3 mm Hg post-losartan vs. 66 ± 1 mm Hg baseline) and P_T (18 ± 1 mm Hg post-losartan vs. 15 ± 1 mm Hg baseline). Values for urine flow rate, sodium excretion, and albumin excretion at two weeks are summarized in Table 4. Baseline values for albuminuria showed that glomerular injury was less advanced than at eight to twelve weeks after ablation. No change was observed in urine flow rate, sodium excretion, or albumin excretion in time control rats. Ang II blockade again caused a significant increase in sodium excretion (2.8 ± 0.5 μEq/min post-losartan vs. 0.3 ± 0.1 μEq/min baseline, $P < 0.05$) while slightly increasing albumin excretion (32 ± 3 μg/min post-losartan vs. 27 ± 4 μg/min baseline).

Losartan dose

The effectiveness of Ang II receptor blockade was first tested by assessing the pressor response to an intravenous bolus infusion of Ang II. The pressor response to 50 ng of Ang II (47 ± 7 mm Hg in time control rats at eight to twelve weeks and 43 ± 5 mm Hg in time control rats at two weeks) was ablated by losartan (1 ± 1 mm Hg in Ang II blockade rats at eight to twelve weeks and 1 ± 1 mm Hg in Ang II blockade rats at two weeks). To further confirm the effectiveness of Ang II receptor blockade, experiments were performed in a separate group of intact rats receiving a continuous infusion of exogenous angiotensin II. Baseline measurements made during infusion of Ang II revealed high values for $\overline{AP}$ and $\overline{\Delta P}$ comparable to those observed in remnant kidney rats (Table 5). Administration of losartan rapidly reduced $\overline{AP}$ and $\overline{\Delta P}$ despite continued infusion of exogenous Ang II. The reduction of $\overline{AP}$, which averaged 45 mm Hg, was associated with a reduction in urine flow rate without a significant change in urine sodium or albumin excretion rate.

Discussion

Renal ablation generally leads to hypertension in the rat. The magnitude of the increase in blood pressure appears to depend on

Table 3. Summary of renal cortical microcirculation studies at two weeks

		Hct	C_A	$\overline{AP}$	GFR	FF	SNGFR	Q_A	P_{GC}	P_T	$\overline{\Delta P}$	K_f
		%	*g/dl*	*mm Hg*	*ml/min*	%	*nl/min*		*mm Hg*			*nl/(s⁻¹ · mm Hg)*
Time control	baseline	48 ± 1	5.7 ± 0.1	137 ± 8	0.70 ± 0.06	0.27 ± 0.03			65 ± 1	16 ± 1	49 ± 1	
(*N* = 6)	post	47 ± 1	5.6 ± 0.1	140 ± 7	0.69 ± 0.06	0.29 ± 0.02	77 ± 6	273 ± 34	65 ± 1	17 ± 1	48 ± 2	0.055 ± 0.005
Ang II blockade	baseline	48 ± 1	5.8 ± 0.1	136 ± 7	0.82 ± 0.03	0.27 ± 0.03			66 ± 1	15 ± 1	50 ± 1	
(*N* = 8)	post	47 ± 1	5.6 ± 0.1	132 ± 6	0.91 ± 0.04[a,b]	0.25 ± 0.02	86 ± 4	339 ± 34	68 ± 3	18 ± 1[a]	50 ± 3	0.051 ± 0.004

Abbreviations are the same as for Table 1.
[a] $P < 0.05$ post vs. baseline
[b] $P < 0.05$ Ang II blockade vs. Time control value for comparable period

Table 4. Urine sodium and albumin excretion at two weeks

		Urine flow rate	Urinary sodium excretion	Urinary albumin excretion
		μl/min	*μEq/min*	*μg/min*
Time control	baseline	12 ± 2	0.5 ± 0.1	36 ± 10
(*N* = 7)	post	11 ± 1	0.5 ± 0.1	36 ± 10
Ang II blockade	baseline	9 ± 1	0.3 ± 0.1	27 ± 4
(*N* = 7)	post	22 ± 2[a,b]	2.8 ± 0.5[a,b]	32 ± 3[a]

[a] $P < 0.05$ post vs. baseline
[b] $P < 0.05$ Ang II blockade vs. Time control

the ablation procedure used and also to vary among individual animals [2, 14]. When present, systemic hypertension is accompanied by glomerular capillary hypertension and associated with progressive development of glomerular injury manifested by proteinuria. The hemodynamic changes can be reversed and the injury prevented by sustained blockade of Ang II activity [1, 4]. These findings suggest that endogenous Ang II increases systemic and glomerular pressure and promotes glomerular injury in the remnant kidney.

The purpose of the current study was to explore the mechanism by which Ang II increases systemic and glomerular pressure following renal ablation. Two previous studies have examined this issue. Rosenberg, Kren and Hostetter [10] found that a peptide Ang II receptor blocker reduced P_{GC} and SNGFR but did not change K_f at four weeks following renal ablation in rats maintained on a synthetic diet with higher protein and lower sodium content than standard laboratory chow. Pelayo, Quan and Shanley [11] found that infusion of a converting enzyme inhibitor and a peptide Ang II receptor blocker reduced P_{GC} and increased K_f so as to maintain SNGFR constant at two weeks after ablation in rats maintained on standard chow. In both these studies, as in the current study, Ang II blockade did not change systemic blood pressure, suggesting that systemic hypertension in the renal ablation model is not caused by direct vasoconstrictor action of Ang II on the peripheral vasculature. However, in the current study, we found that Ang II blockade with the non-peptide receptor antagonist losartan did not reduce P_{GC} or ΔP. No explanation for the differing effects of Ang II blockade on P_{GC} and K_f in the current study and in the studies of Rosenberg et al [10] and Pelayo et al [11] is readily apparent. The major effect of Ang II blockade observed in the current study was to increase urine sodium excretion. Sodium excretion was not assessed in the previous studies of the effect of Ang II blockade on remnant kidney function [10, 11]. The current findings, however, suggest that a major effect of endogenous Ang II in the remnant kidney is to promote sodium retention. Other studies suggest that sodium retention could account for systemic and glomerular hypertension in the remnant kidney model. Sustained sodium retention is a known cause of systemic hypertension [15]. Moreover, in the remnant kidney, autoregulation is impaired, so that increases in systemic pressure are accompanied by increases in glomerular pressure [16, 17]. Thus, Ang II could cause remnant glomerular hypertension indirectly by promoting sodium retention. Such an effect of Ang II on glomerular pressure would not be reversible by acute Ang II blockade, in accord with the results of the current study.

The suggestion that Ang II causes hypertension by promotion of sodium retention rather than by direct vasoconstriction is consistent with the finding that ECF volume is increased while circulating renin activity is low or normal in the renal ablation model [18–23]. Recent studies have shown further that circulating angiotensin II levels are normal in renal ablated rats with hypertension [24]. Normal circulating levels of Ang II would not be expected to cause systemic vasoconstriction but could cause sodium retention [7]. It should be noted that the sodium retaining effect of Ang II may be exaggerated in rats subjected to renal ablation. Because their GFR is reduced these animals must increase a fractional sodium excretion to approximately four times normal to excrete the daily sodium load. The "normal" circulating Ang II activity observed by Kuczera et al [24] following renal ablation could be inappropriately high in this setting, and losartan could cause natriuresis by blocking this Ang II activity. Recent studies suggest that endogenous Ang II tone is sufficient to promote proximal sodium reabsorption in intact rats [6, 25]. Blockade of Ang II activity with losartan has been shown to increase sodium excretion in these animals [25, 26]. In the current study, intact rats whose blood pressure was increased by infusion of Ang II did not exhibit an increase in sodium excretion when Ang II activity was blocked and blood pressure was reduced by losartan. This finding is consistent with previous reports that changes in systemic pressure offset the direct tubular effect of Ang II on sodium reabsorption during exogenous Ang II infusion [27].

An alternate possibility is that losartan causes natriuresis by blocking the activity of Ang II generated within the kidney. Measurement of intrarenal Ang II levels has not yet been accomplished in rats subjected to renal ablation. Two studies, however, have examined renal content of renin and renin message in this model [22, 28]. Both found an increase in renin and renin message adjacent to infarct scars and a reduction in renin and

Table 5. Effect of acute angiotensin II blockade in normal rats receiving intravenous angiotensin II

	Hct	C_A	$\overline{AP}$	GFR	FF	P_{GC}	P_T	$\overline{\Delta P}$	U_{vol}	$U_{Na}V$	$U_{Alb}V$
	%	g/dl	mm Hg	ml/min	%	mm Hg			μl/min	μEq/min	μg/min
Baseline	48 ± 1	5.9 ± 0.1	148 ± 6	1.1 ± 0.02	0.33 ± 0.02	70 ± 2	14 ± 1	56 ± 2	8 ± 1	0.4 ± 0.1	3 ± 1
Post	49 ± 1	5.7 ± 0.1	103 ± 4[a]	1.0 ± 0.07	0.20 ± 0.02[a]	51 ± 1[a]	14 ± 1	37 ± 1[a]	4 ± 1[a]	0.3 ± 0.1	3 ± 1

Abbreviations are same as for Table 1 and: U_{vol}, urine flow rate; $U_{Na}V$, urinary sodium excretion; $U_{Alb}V$, urinary albumin excretion.
[a] $P < .05$ post vs. baseline

renin message away from these scars. These findings are consistent with the hypothesis that renin produced in areas adjacent to infarction causes hypertension and suppresses the production of renin in the remainder of the kidney. Measurement of renin and renin message, however, does not provide a direct index of renin release. Moreover, there is increasing reason to believe that local Ang II activity at different sites within the kidney does not depend entirely on renin release from juxtaglomerular cells. Recent studies suggest that the proximal tubule possesses the elements required for local generation of Ang II, including renin, angiotensinogen, and converting enzyme [29–31]. Levels of Ang II in proximal tubule fluid have been found to be much higher than those in the kidney as a whole, suggesting the tubule secretes Ang II into its lumen [32]. The activity of the proximal tubule renin angiotensin system could theoretically be altered in the remnant kidney without a parallel alteration in the circulating renin-angiotensin system. The recent finding of an increase in glomerular angiotensinase A in renal ablated rats suggests further that Ang II activity at specific sites in the remnant kidney could be affected by changes in Ang II degradation rate [33]. Finally, it should be noted that in one study of renin activity in the remnant kidney, glomerular renin and renin message were found to be increased, while total renin in non-infarct areas was reduced [21]. The current results would suggest that renin activity in remnant glomeruli, though it could contribute to the development of glomerular injury, is not sufficient to cause a direct, Ang II mediated increase in glomerular pressure or a reduction in K_f.

The current study further suggests that endogenous Ang II does not have an acutely reversible effect on remnant glomerular permselectivity. Urinary albumin excretion was unchanged by losartan at 8 to 12 weeks after ablation. A slight increase in urine albumin excretion accompanied a similar slight increase in GFR following losartan administration at two weeks after ablation. In normal rats, infusion of modest doses of exogenous Ang II reduces K_f and infusion of large doses of Ang II may cause proteinuria [8, 34]. The structural changes responsible for these acute alterations in function have not been identified. Steiner and Blantz [9] have shown, however, that the reduction in K_f caused by exogenous Ang II in normal rats can rapidly be reversed by Ang II blockade. This finding was confirmed in experiments performed to establish the adequacy of the losartan dose used in the current study. In remnant kidney rats, losartan did not cause an acute increase in K_f or an acute reduction in albuminuria. Previous studies in the remnant kidney rat have shown that sustained Ang II blockade increases K_f and largely prevents development of albuminuria. These studies have shown further that after albuminuria is established, one week of Ang II blockade increases K_f and reduces the albumin excretion rate by restoring glomerular size-selectivity toward normal [4, 5]. Together, these results suggest that endogenous Ang II contributes to the progressive development of size-selectivity defects in remnant glomeruli but that the rate at which macromolecules escape through such defects is not acutely dependent on Ang II activity. These results are consistent with recent studies in human renal disease, which have shown that Ang II converting enzyme reduces protein excretion rate only over a period of days, and that the reduction of protein excretion rate achieved by converting enzyme inhibition is not reversed by acute administration of Ang II [35, 36].

While the current data are consistent with the hypothesis that the major effect of Ang II in the remnant kidney is to promote sodium retention, it should be acknowledged that other interpretations of these data are possible. First, it is always difficult to establish that the activity of an endogenous hormone has been completely blocked. The dose of losartan used in the current study was sufficient to reverse the increase in $\overline{\Delta P}$ and the reduction in K_f caused by exogenous Ang II. The possibility that endogenous Ang II reaches a different population of receptors and has actions which are not blocked by losartan, however, cannot be excluded. Moreover, sustained Ang II activity may cause changes other than sodium retention which are not acutely reversible by Ang II receptor blockade. Changes in vascular structure caused by the combination of increased blood pressure and Ang II activity have been blamed for the occasional persistence of hypertension following removal of the clipped kidney in rats with two-kidney one-clip Goldblatt hypertension. Ang II may also simulate production of other vasoactive hormones, including endothelin, which has been found to have increased activity in the remnant kidney [37]. The possibility that Ang II stimulates endothelin production is of particular interest since the long-acting vasoconstrictor effects of endothelin might not be acutely reversed by Ang II receptor blockade.

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References

1. Anderson S, Meyer TW, Rennke HG, Brenner BM: Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *J Clin Invest* 76:612–619, 1985
2. Bidani AK, Mitchell KD, Schwartz MM, Navar LG, Lewis EJ: Absence of glomerular injury or nephron loss in a normotensive rat remnant kidney model. *Kidney Int* 38:28–38, 1990
3. Meyer TW, Rennke HG: Progressive glomerular injury after limited renal infarction in the rat. *Am J Physiol* 254:F856–F862, 1988

4. LAFAYETTE RA, MAYER G, PARK SK, MEYER TW: Angiotensin II receptor blockade limits glomerular injury in rats with reduced renal mass. *J Clin Invest* 90:766–771, 1992
5. MAYER G, LAFAYETTE RA, OLIVER J, DEEN WM, MYERS BD, MEYER TW: Effects of angiotensin II receptor blockade on remnant glomerular permselectivity. *Kidney Int* 43:346–453, 1993
6. COGAN MG, LIU F-Y, WONG PC, TIMMERMANS PS: Comparison of inhibitory potency by nonpeptide angiotensin II receptor antagonists PD123177 and DuP 753 on proximal nephron and renal transport. *J Pharm Exper Ther* 259:687–691, 1991
7. SEIDELIN PH, MCMURRAY JJ, STRUTHERS AD: Mechanisms of the antinatriuretic action of physiological doses of angiotensin II in man. *Clin Sci* 76:653–658, 1989
8. BLANTZ RC, KONNEN KS, TUCKER BJ: Angiotensin II effects upon the glomerular microcirculation and ultrafiltration coefficient of the rat. *J Clin Invest* 57:419–434, 1976
9. STEINER RW, BLANTZ RC: Acute reversal by saralasin of multiple intrarenal effects of angiotensin II. *Am J Physiol* 237:F386–F391, 1979
10. ROSENBERG ME, KREN SM, HOSTETTER TH: Effect of dietary protein on the renin-angiotensin system in subtotally nephrectomized rats. *Kidney Int* 38:240–248, 1990
11. PELAYO JC, QUAN AH, SHANLEY PF: Angiotensin II control of the renal microcirculation in rats with reduced renal mass. *Am J Physiol* 258:F414–422, 1990
12. WONG PC, PRICE WA JR, CHIU AT, DUNCIA JV, CARINI DJ, WEXLER RR, JOHNSON AL, TIMMERMANS PBMWM: Hypotensive action of DuP 753, an angiotensin II antagonist, in spontaneously hypertensive rats. Nonpeptide angiotensin II receptor antagonists: X. *Hypertension* 15:459–468, 1990
13. DEEN WM, TROY JL, ROBERTSON CR, BRENNER BM: Dynamics of glomerular ultrafiltration in the rat. IV. Determination of the ultrafiltration coefficient. *J Clin Invest* 52:1500–1508, 1973
14. ROBSON AM, MOR J, ROOT ER, JAGER BV, SHANKEL SW, INGELFINGER JR, KIENSTRA RA, BRICKER NS: Mechanism of proteinuria in nonglomerular disease. *Kidney Int* 16:416–429, 1979
15. GUYTON AC: Dominant role of the kidneys and accessory role of whole-body autoregulation in the pathogenesis of hypertension. *Am J Hypertens* 2:575–585, 1989
16. GRIFFIN KA, BIDANI AK, PICKEN M, ELLIS VR, CHURCHILL PC: Prostaglandins do not mediate impaired autoregulation or increased renin secretion in remnant rat kidneys. *Am J Physiol* 263:F1057–F1062, 1992
17. PELAYO JC, WESTCOTT JY: Impaired autoregulation of glomerular capillary hydrostatic pressure in the rat remnant nephron. *J Clin Invest* 88:101–105, 1991
18. YLITALO P, GROSS F: Hemodynamic changes during the development of sodium-induced hypertension in subtotally nephrectomized rats. *Acta Physiol Scand* 106:447–455, 1979
19. KAYSEN GA, WATSON JB: Mechanism of hypoalbuminemia in the 7/8-nephrectomized rat with chronic renal failure. *Am J Physiol* 243:F372–F378, 1982
20. JACKSON B, HODSMAN P, JOHNSTON CI: Changes in the renin-angiotensin system, exchangeable body sodium, and plasma and atrial content of atrial natriuretic factor during evolution of chronic renal failure in the rat. *Am J Hypertens* 1:298–300, 1988
21. ROSENBERG ME, CORREA-ROTTER R, INAGAMI T, KREN SM, HOSTETTER TH: Glomerular renin synthesis and storage in the remnant kidney in the rat. *Kidney Int* 40:677–683, 1991
22. PUPILLI C, CHEVALIER RL, CAREY RM, GOMEZ RA: Distribution and content of renin and renin mRNA in remnant kidney of adult rat. *Am J Physiol* 263:F731–F738, 1992
23. GRETZ N, WALDHERR R, STRAUCH M: The remanant kidney model, in *Experimental and Genetic Rat Models of Chronic Renal Failure*, edited by GRETZ N, STRAUCH M, Basel, Karger, 1993, pp 1–28
24. KUCZERA M, HILGERS KF, LISSON C, GANTEN D, HILGENFELDT U, RITZ E, MANN JFE: Local angiotensin formation in hindlimbs of uremic hypertensive and renovascular hypertensive rats. *J Hypertens* 9:41–48, 1991
25. XIE M-H, LIU F-Y, WONG PC, TIMMERMANS PBMWM, COGAN MG: Proximal nephron and renal effects of DuP 753, a nonpeptide angiotensin II receptor antagonist. *Kidney Int* 38:473–479, 1990
26. FENOY FJ, MILICIC I, SMITH RD, WONG PC, TIMMERMANS PBMWM, ROMAN R: Effects of DuP 753 on renal function of normotensive and spontaneously hypertensive rats. *Am J Hypertens* 4:321S-326S, 1991
27. OLSEN ME, HALL JE, MONTANI J-P, GUYTON AC, LANGFORD HG, CORNELL JE: Mechanisms of angiotensin II natriuresis and antinatriuresis. *Am J Physiol* 249:F299–F307, 1985
28. CORREA-ROTTER R, HOSTETTER TH, ROSENBERG ME: Effect of dietary protein on renin and angiotensinogen gene expression after renal ablation. *Am J Physiol* 262:F631–F638, 1992
29. INGELFINGER JR, ZUO WM, FON EA, ELLISON KE, DZAU VJ: In situ hybridization evidence for angiotensinogen messenger RNA in the rat proximal tubule. An hypothesis for the intrarenal renin angiotensin system. *J Clin Invest* 85:417–423, 1990
30. MOE OW, UJIIE K, STAR RA, MILLER RT, WIDELL J, ALPERIN RJ, HENRICH WL: Renin expression in renal proximal tubule. *J Clin Invest* 91:774–779, 1993
31. MOE OW, ALPERN RJ, HENRICH WL: The renal proximal tubule renin-angiotensin system. *Semin Nephrol* 13:552–557, 1993
32. BRAAM B, MITCHELL KD, FOX J, NAVAR LG: Proximal tubular secretion of angiotensin II in rats. *Am J Physiol* 264:F891–F898, 1993
33. WOLF G, THAISS F, SCHERBERICH JE, SCHOEPPE W, STAHL RAK: Glomerular angiotensinase A in the rat: Increase of enzyme activity following renal ablation. *Kidney Int* 38:862–868, 1990
34. OLIVETTI G, KITHIER K, GIACOMELLI F, WIENER J: Characterization of glomerular permeability and proteinuria in acute hypertension in the rat. *Kidney Int* 25:599–607, 1984
35. HEEG JE, DEJONG PE, VAN DER HEM GK, DE ZEEUW D: Angiotensin II does not acutely reverse the reduction of proteinuria by long-term ACE inhibition. *Kidney Int* 40:734–741, 1991
36. GANSEVOORT RT, DE ZEEUW D, DE JONG PE: Dissociation between the course of the hemodynamic and antiproteinuric effects of angiotensin I converting enzyme inhibition. *Kidney Int* 44:579–584, 1993
37. ORISIO S, BENIGNI A, BRUZZI I, CORNA D, PERICO N, ZOJA C, BENATTI L, REMUZZI G: Renal endothelin gene expression is increased in remnant kidney and correlates disease progression. *Kidney Int* 43:354–358, 1993